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Rostrum: Altered T-cell receptor signaling in the pathogenesis of allergic disease

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Abstract

Mounting evidence from animal models has demonstrated that alterations in T-cell receptor signaling alone can lead to dramatically skewed differentiation of naïve T-cells into Th2 cells, to Th2 effector functions, and to Th2-related diseases. There is significant potential relevance of these observations to human disease. Specifically, a number of immunodeficiencies which are associated with atopic disease may have atopy as a manifestation due to aberrant TCR signaling. It is therefore important to attempt to identify a role for defects in T-cell receptor signaling in the pathogenesis of common atopic diseases.

Keywords

T-cell Receptor; Th2; Atopy

Introduction

“A little learning is a dangerous thing; drink deep, or taste not the Pierian spring: there shallow draughts intoxicate the brain, and drinking largely sobers us again.” Alexander Pope made the statement regarding scholarship, but the truth of the matter is that same maxim can often be applied in biological systems as well. In particular, T-cell receptor signaling may provide an instructive example of this, and understanding this example may go a long way to understanding the pathophysiology of atopic disease and, to some extent, immunodeficiency. Nearly 15 years ago, Bottomly, O'Garra and others began an area of work which showed that weak signaling through a T-cell receptor could lead an undifferentiated naïve CD4⁺ T cell to acquire a Th2 phenotype, independent of the exogenous cytokines present at the time of priming. Since then, other pieces of evidence have been added to paint a picture where indeed, a weak TCR (T-cell receptor) signal might be worse than no signal at all. Most of this work has largely been done in mouse models. It is therefore of significant interest to determine the extent to which the phenomenon of attenuated signals through the TCR might predispose patients to atopic disease.

Th2 phenotypes due to altered peptide-MHC/TCR interactions

Peptides that interact strongly with the TCR of naïve CD4 T-cells favor Th1 and Th17 cell differentiation while low affinity peptide-MHC/TCR interactions favor Th2 effector T cell

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differentiation (1–3). In addition, increased antigen dose—mimicking increased receptor occupancy, has shown to lead to IFN γ producing cells where as lower antigen dose elicits GATA-3 expression and IL-4 producing cells (4). The well-described Th2 responses to schistosome egg antigen (SEA) have recently been demonstrated to be, in part, due to the ability of SEA component Omega-1 to depress the capacity for TCR-DC interactions by affecting actin polymerization and thereby weakening cell adherence and synapse formation, without affecting MHC expression. Importantly, the Th2 induction was independent of exogenous IL-4 (5,6). Finally, poor antigen processing of a cognate antigen can lead transgenic T-cells specific for that antigen to cause a Th2 autoimmune gastritis (7).

There is also evidence that within polyclonal populations, TCR affinity contributes to effector fate as well. It has been suggested that longer complementarity determining region (CDR) 3 regions in the TCR β chain predispose polyclonal naïve T-cells to differentiate into Th2 cells under polarizing conditions due to bulkier CDR3 loops which effectively weaken the interactions of the TCR with other critical binding regions in the MHC class II-peptide complex (8). We have recently demonstrated that within a polyclonal population, removal of cells with high affinity for a given antigen allows lower affinity cells to proliferate, resulting in the generation of a Th2 response. In the same system, IL-4 producing antigen-specific clones generated from a polyclonal population tended to have fewer ideal CDR3 motifs (and hence less presumed functional avidity) specific for the antigen than those which made less IL-4 (9). These studies argue that weak TCR specificities for a given antigen can lead responding cells to acquire a Th2 phenotype when higher affinity cells are absent. The low receptor occupancy caused by weak TCR affinity for peptide-MHC complexes leads to the same responses seen with weak agonists and/or low peptide doses.

Whether due to weak peptide agonists, low peptide concentration, or low affinity of the naïve TCR for a given antigen, the mechanism which leads these primed cells to develop a Th2 response is not fully understood. It does appear that the strength of the TCR signal influences downstream signaling events, which in turn dictate transcription factor and cytokine gene transcription (10). Presumably, the association of TCR affinity with the Th2 phenotype is due both to the active induction of Th2 cytokines, which can occur in the absence of exogenous IL-4, as well as the failure to inhibit production of Th2 cytokines. This effect can occur both directly within the cell, and indirectly by failing to produce cytokines which might inhibit Th2 differentiation. Any early IL-4 production can then amplify Th2 differentiation states in an autocrine fashion. In altered peptide ligand stimulated cells, a lack of Zap70, LAT and phospholipase C- γ 1 phosphorylation and weak calcium mobilization is believed to lead to Th2 generation. Decreased calcium signaling has been shown to lead to increased NFATc to NFATp ratios that induce IL-4 transcription and subsequently amplify Th2 effector cell development through IL-4 receptor mediated STAT6 activation and GATA-3 expression (1). It has also been observed that low concentration peptide stimulation in mice fails to induce phosphorylation of ERK(11), while under high peptide concentration, cells treated with an inhibitor of ERK phosphorylation have upregulated GATA-3 and IL-4 expression-- implying that ERK activation normally represses GATA-3 transcription with TCR activation (4). In addition to decreased calcium flux and ERK phosphorylation, Th2 responses have also been associated with diminished JNK and NF κ B activation (12). It should be noted that IL-2 is required for GATA-3 induction, and so weak signals might not always lead to Th2 induction when, due to the weak TCR signal, insufficient IL-2 is produced (11).

In Vivo Evidence For Atopic phenotypes Due To Altered Signaling Downstream From TCR

In addition to these TCR transgenic and antigen-specific models of TCR signaling strength, further support for the connection between weak TCR signals and Th2 responses comes from TCR signaling molecule mutants that lead to marked Th2 phenotypes. Spontaneous mutants, and mutants derived from mouse colonies exposed to the mutagen N-ethyl-N-nitrosourea (ENU) during breeding, have been generated which are associated with striking Th2 phenotypes including elevated IgE, atopic dermatitis, eosinophilia and Th2 cytokine production. In a number of these strains, Th2 skewing appears to be caused by mutations in TCR signaling molecules, including ZAP70, LAT and Carma1. These mutations do not generally lead to absence of the protein, but rather altered or hypomorphic (decreased activity) functioning. It would be expected that these mutations resulting in the partial defect in TCR signaling would lead to decreased TCR activity, but actually, cytokine production is aberrant, instead of absent. Interestingly, mutations in these proteins where signaling is completely lost do not lead to the same disease state thus suggesting weak signaling, as a result of partial loss-of-function allele, can be more pathogenic than absent signaling due to a null allele (12–17).

For example, early activation through the TCR involves zeta chain-associated protein kinase (Zap) 70 phosphorylation. Two separate reports identify hypomorphic point mutations in ZAP70 that lead to increased plasma IgE concentrations. Interestingly, a single point mutation in the C-terminal SH2 domain can lead to autoimmune arthritis, whereas a point mutation in the kinase domain associates with T-cell deficiency along with increased IgE production suggesting that the location and type of mutation determines the resulting phenotype (14,18,19). The observed phenotypic differences may be due to differing degrees or forms of zap70 functional impairment which permit varying degrees of Th1 or Th2 activation. It is not entirely clear whether these differences are simply in the quantity of signal propagated by zap70, or whether there are qualitative differences in signaling as well which lead to such disparate phenotypes. The Th2 phenotype in the hypomorphic mutation in ZAP 70 may result from TCR stimulation due to defective activation of inhibitory pathways (17), while partially compromised TCR signaling leading to non-Th2 autoimmune disease can be due to both compromised negative selection and regulatory T-cell (Treg) differentiation. In contrast to these hypomorphic mutants, ZAP70 null mutants have normal levels of IgE, but are characterized by severe immunodeficiency. This fine line between lack of function, hypomorphic function leading to Th2 disease, hypomorphic disease leading to other types of immunodysregulation, and a balanced T cell response, shows that signals need to be in just the right range to lead to Th2 pathology. It also speaks to the complexity of effector and regulatory networks, and how mutations can affect both types with complex results.

The linker for activation of T cells (LAT) is phosphorylated by ZAP70 that initiates the recruitment of signaling molecules for T cell activation. A single amino acid substitution in LAT (Y136F) diminishes the docking and activation of PLC γ , but preserves the docking of other signaling molecules. As a result, calcium signaling is decreased, but ERK signaling is maintained. The compromised TCR signaling leads to uncontrolled CD4 T-cell proliferation resulting in increased frequency of Th2 effectors, tissue eosinophilia, and elevated serum IgE. The increased CD4 T-cell proliferation triggers B cell activation resulting in hypergammaglobulinemia IgG1 and IgE. Negative regulatory signals are defective in the Y136F LAT mutation, leading to a loss of proper control of TCR signaling and an increase in Th2 phenotype. Mutation of LAT only in cells which have left the thymus can lead to potent Th2 effector cells as well, indicating the phenotype is not dependent on thymic maturation defects. (15). The single amino acid substitution in LAT leads to autoimmunity

as well presumably due to a stronger affinity for self and impaired negative selection, and these mice have decreased T regulatory cells (20–22). The LAT mutant mice highlight the importance of determining whether some defects in TCR signaling that permit a Th2 phenotype are due to poor Treg function, since proper TCR activation is important for Treg function (23). Additionally, FoxP3-deficient mice and humans with IPEX (Immunodysregulation, polyendocrinopathy, enteropathy, X-linked) have a strong Th2 predilection amongst effector cells (in addition to aberrant production of other helper cytokines), as well as prominent Th2 pathology, suggesting a critical role for FoxP3 (and presumably Tregs) in the suppression of Th2 disease (24).

Further downstream of LAT activation is the complex containing a member of the membrane-associated guanylate kinase (MAGUK) family of proteins, Carma1, that leads to NFkB activation. A hypomorphic mutation in Carma1 alters a conserved leucine in the coiled-coil domain leading to high serum levels of IgE and dermatitis on the ear and neck (12). Carma1 plays a role in mediating effects of CD28 including up-regulation of IL-2 receptors and promotion of cell division. Proximal TCR signaling is not affected since comparable total tyrosine phosphorylation and calcium fluxes are observed. Interestingly, both Carma1 null and point mutants have defective JNK and NFkB signaling however only the point mutation leads to elevated serum IgE and atopic dermatitis, presumably due to the residual function which permits activation of Th2 cytokines (12,13).

As mentioned above, weak affinity of the TCR repertoire for a given antigen can also lead to Th2 phenotypes. Marked alterations of in vivo TCR repertoires might therefore be expected, in some cases, to lead to a Th2 phenotype, despite otherwise normal TCR signaling genes, and indeed there are a number of examples of this phenomenon. One of the best examples of this is Omenn Syndrome (OS). OS is a form of severe combined immunodeficiency (SCID) which is characterized by oligoclonal T-cell expansion with marked skewing of the TCR repertoire, multi-organ histiocytosis, elevated IgE, eosinophilia and exfoliative dermatitis. It can be caused by hypomorphic mutations in genes which can also cause SCID, but for reasons which are not clear, has the additional phenotype of this oligoclonal T-cell expansion and Th2 pathology. The reasons for the breakdown in tolerance in OS may include lack of thymic AIRE expression (25) and decreased FoxP3+ Tregs, as suggested by recent mouse models of OS (26).

It should be noted that even with normal thymic development and normal Treg formation, a limited repertoire of CD4 T-cells from wild-type mice can lead to a severe multi-organ lymphopenia-induced Th2 inflammatory disease (27). One explanation for this finding is that the lymphopenic state may allow for TCR with relatively low affinity to interact and respond to antigens which, in more lymphoreplete settings, might never occur due to competition (9). Additionally, the lack of a diverse TCR repertoire among the FoxP3+ Tregs appears responsible for the Th2 disease, as transfer of FoxP3+ Tregs with more diverse TCR repertoires can prevent the disease, while equal numbers of Tregs derived from a more narrow repertoire does not (27).

Clinical evidence for altered TCR signaling in atopic disease

Despite evidence from hypomorphic mutations of TCR signaling molecules in mice characteristic of Th2 phenotypes, to date, less observed evidence exists for such a pathogenic pathway in humans. Most humans with ZAP70 mutations have a SCID-like phenotype. However, there is a description of a hypomorphic mutation of ZAP70 associated with elevated IgE levels, lower number of CD8 and CD4 T cells and poorly functional T cells in one patient. Despite quantitatively compromised TCR signaling, there are no signs of autoimmunity, as observed in the C-terminal SH2 domain ZAP70 mutant mouse. This

patient's phenotype is similar to the kinase domain ZAP70 mouse mutant (14,16). Another patient with ZAP70 mutation, but with some residual protein expression, has been reported to have a phenotype similar to Omenn's Syndrome with markedly elevated IgE, histiocytosis and T-cell lymphocytosis. It is not known whether the phenotype observed was similar to Omenn syndrome pathology in that the limited TCR repertoire leads to disease or whether this Th2 phenotype was due to impaired Zap70 signaling mimicking the mouse models of ZAP70 hypomorphic mutations (28). It should be noted that in contrast to the oligoclonal expansions seen in OS, the Zap70 mutant mice were actually lymphopenic, perhaps suggesting a separate pathology.

A number of other immunodeficiencies exist which are often associated with atopic phenotypes. Wiskott-Aldrich syndrome and DOCK8 deficiency are associated with elevated IgE, severe atopic dermatitis and other allergic disease (29). Both diseases are caused by mutations in proteins that participate in actin cytoskeleton rearrangement and immune synapse formation and as such might be anticipated to be involved in TCR signaling (30–32). The association of alterations of cytoskeleton rearrangement in these diseases with a Th2 response may well be similar to the effects of omega-1 described earlier. The only direct study of weakened TCR signaling and Th2 phenotype in either disease can be found in the WASP-/- mouse which actually showed a defect in Th2 responses—however significant differences in Th2 phenotypes between the mouse and humans preclude drawing definitive conclusions on this issue.

In addition to Omenn Syndrome and IPEX, both described above, another example of an immunodeficiency associated with Th2 pathology is the autosomal dominant Hyper-IgE Syndrome, caused by STAT3 mutations. Patients have eczema and markedly elevated IgE, as well as fungal and staphylococcal infections and other non-immunologic phenotypes, however STAT3 is largely a cytokine signaling molecule, and few reports link it directly to TCR signaling (33).

Conclusion

In exploring these concepts, a question worth asking is: Why does the immune system have this strange capacity to produce Th2 cytokines with weak signals, and have other cytokines depend on stronger signals? One potential hypothesis could be that some pathogens try to evade clearance by weakening TCR signals, and so this alternate cytokine program evolved, one which had less tissue destruction capacity than, say, IL-17 or IFN γ , but which could eradicate some pathogens. The SEA omega-1 example illustrates this concept well (5). It might also be posited that the overwhelming antigen load presented by large parasites lead to the need to have decreased TCR signal strength when encountering parasites in order to avoid overwhelming the immune system. Th2 immunity may have evolved as a result of this adaptation: Weak TCR signaling gives rise to Th2 responses and IgE production, which is an effective antibody response to eradicate parasites that are too large to be phagocytosed. Another possibility is that the observation that weakened TCR signaling leads to Th2 pathology is not an evolved mechanism which confers an advantage in some cases. Rather, it is also possible that primed naïve T-cells have complicated counter-regulatory networks which are integrated to decide Thelper fate. These networks lead to a system where naïve cells have the capacity to easily produce many different types of cytokines, all of which are normally well suppressed, but also relatively easy to induce with the right external signals, including signal strength. Rare disruptions of these networks—in this case of Th2 cells, due to weakened signal strength-- can therefore lead to aberrant absence of suppression.

Regardless of the evolutionary explanation, naïve CD4 T-cells have cell-intrinsic capacities to become Th2 cells and be predisposed to causing atopic pathology. One type of

mechanism includes low peptide-TCR affinity due to poor antigen presentation, weak TCR-DC interactions, and skewed TCR repertoires which leave only lower affinity clones specific for given antigens. The second type is poor TCR signaling due to mutations in TCR signaling molecules. These phenomena have yet to be convincingly demonstrated in human disease, but merit exploration in both known immunodeficiencies associated with atopic disease, as well as more common severe atopic disease in the absence of other significant immunopathology. Devising strategies to measure antigen or allergen-specific TCR affinity, and to screen for hypomorphic TCR signaling mutations are important tools which will be necessary to explore this question.

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Abbreviations

TCR	T-cell receptor
Th	T-helper
MHC	Major histocompatibility complex
(SEA)	schistosome egg antigen

References

1. Boutin Y, Leitenberg D, Tao X, Bottomly K. Distinct biochemical signals characterize agonist- and altered peptide ligand-induced differentiation of naive CD4+ T cells into Th1 and Th2 subsets. *J Immunol* Dec 15;1997 159(12):5802–9. [PubMed: 9550376]
2. Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A. The effect of antigen dose on CD4+ T helper cell phenotype development in a T cell receptor-alpha beta-transgenic model. *J Exp Med* Nov 1;1995 182(5):1579–84. [PubMed: 7595228]
3. Gomez-Rodriguez J, Sahu N, Handon R, Davidson TS, Anderson SM, Kirby MR, et al. Differential expression of interleukin-17A and -17F is coupled to T cell receptor signaling via inducible T cell kinase. *Immunity* Oct 16;2009 31(4):587–97. [PubMed: 19818650]
4. Yamane H, Zhu J, Paul WE. Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. *J Exp Med* Sep 19;2005 202(6):793–804. [PubMed: 16172258]
5. Steinfelder S, Andersen JF, Cannons JL, Feng CG, Joshi M, Dwyer D, et al. The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). *J Exp Med* Aug 3;2009 206(8):1681–90. [PubMed: 19635859]
6. Everts B, Perona-Wright G, Smits HH, Hokke CH, van der Ham AJ, Fitzsimmons CM, et al. Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. *J Exp Med* Aug 3;2009 206(8):1673–80. [PubMed: 19635864]
7. Levin D, DiPaolo RJ, Brinster C, Revilla MJ, Boyd LF, Teyton L, et al. Availability of autoantigenic epitopes controls phenotype, severity, and penetrance in TCR Tg autoimmune gastritis. *Eur J Immunol* Dec;2008 38(12):3339–53. [PubMed: 19039784]
8. Boyton RJ, Zaccari N, Jones EY, Altmann DM. CD4 T cells selected by antigen under Th2 polarizing conditions favor an elongated TCR alpha chain complementarity-determining region 3. *J Immunol* Feb 1;2002 168(3):1018–27. [PubMed: 11801634]
9. Milner JD, Fazilleau N, McHeyzer-Williams M, Paul W. Cutting Edge: Lack of High Affinity Competition for Peptide in Polyclonal CD4+ Responses Unmasks IL-4 Production. *J Immunol*. May 21;2010

10. Brogdon JL, Leitenberg D, Bottomly K. The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naive CD4+ T cells. *J Immunol* Apr 15;2002 168(8):3825–32. [PubMed: 11937535]
11. Jorritsma PJ, Brogdon JL, Bottomly K. Role of TCR-induced extracellular signal-regulated kinase activation in the regulation of early IL-4 expression in naive CD4+ T cells. *J Immunol* Mar 1;2003 170(5):2427–34. [PubMed: 12594266]
12. Jun JE, Wilson LE, Vinuesa CG, Lesage S, Blery M, Miosge LA, et al. Identifying the MAGUK protein Carma-1 as a central regulator of humoral immune responses and atopy by genome-wide mouse mutagenesis. *Immunity* Jun;2003 18(6):751–62. [PubMed: 12818157]
13. Hara H, Wada T, Bakal C, Kozieradzki I, Suzuki S, Suzuki N, et al. The MAGUK family protein CARD11 is essential for lymphocyte activation. *Immunity* Jun;2003 18(6):763–75. [PubMed: 12818158]
14. Jakob T, Kollisch GV, Howaldt M, Bewersdorff M, Rathkolb B, Muller ML, et al. Novel mouse mutants with primary cellular immunodeficiencies generated by genome-wide mutagenesis. *J Allergy Clin Immunol* Jan;2008 121(1):179–84. e7. [PubMed: 17767948]
15. Mingueneau M, Roncagalli R, Gregoire C, Kissenpfennig A, Miazek A, Archambaud C, et al. Loss of the LAT adaptor converts antigen-responsive T cells into pathogenic effectors that function independently of the T cell receptor. *Immunity* Aug 21;2009 31(2):197–208. [PubMed: 19682930]
16. Picard C, Dogniaux S, Chemin K, Maciorowski Z, Lim A, Mazerolles F, et al. Hypomorphic mutation of ZAP70 in human results in a late onset immunodeficiency and no autoimmunity. *Eur J Immunol* Jul;2009 39(7):1966–76. [PubMed: 19548248]
17. Siggs OM, Miosge LA, Yates AL, Kucharska EM, Sheahan D, Brdicka T, et al. Opposing functions of the T cell receptor kinase ZAP-70 in immunity and tolerance differentially titrate in response to nucleotide substitutions. *Immunity* Dec;2007 27(6):912–26. [PubMed: 18093540]
18. Sakaguchi N, Takahashi T, Hata H, Nomura T, Tagami T, Yamazaki S, et al. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* Nov 27;2003 426(6965):454–60. [PubMed: 14647385]
19. Yoshitomi H, Sakaguchi N, Kobayashi K, Brown GD, Tagami T, Sakihama T, et al. A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J Exp Med* Mar 21;2005 201(6):949–60. [PubMed: 15781585]
20. Aguado E, Richelme S, Nunez-Cruz S, Miazek A, Mura AM, Richelme M, et al. Induction of T helper type 2 immunity by a point mutation in the LAT adaptor. *Science* Jun 14;2002 296(5575):2036–40. [PubMed: 12065839]
21. Koonpaew S, Shen S, Flowers L, Zhang W. LAT-mediated signaling in CD4+CD25+ regulatory T cell development. *J Exp Med* Jan 23;2006 203(1):119–29. [PubMed: 16380508]
22. Sommers CL, Park CS, Lee J, Feng C, Fuller CL, Grinberg A, et al. A LAT mutation that inhibits T cell development yet induces lymphoproliferation. *Science* Jun 14;2002 296(5575):2040–3. [PubMed: 12065840]
23. Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* Jul 20;1998 188(2):287–96. [PubMed: 9670041]
24. Lin W, Truong N, Grossman WJ, Haribhai D, Williams CB, Wang J, et al. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J Allergy Clin Immunol* Nov;2005 116(5):1106–15. [PubMed: 16275384]
25. Poliani PL, Facchetti F, Ravanini M, Gennery AR, Villa A, Roifman CM, et al. Early defects in human T-cell development severely affect distribution and maturation of thymic stromal cells: possible implications for the pathophysiology of Omenn syndrome. *Blood* Jul 2;2009 114(1):105–8. [PubMed: 19414857]
26. Marrella V, Poliani PL, Casati A, Rucci F, Frascoli L, Gougeon ML, et al. A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome. *J Clin Invest* May;2007 117(5):1260–9. [PubMed: 17476358]
27. Milner JD, Ward JM, Keane-Myers A, Paul WE. Lymphopenic mice reconstituted with limited repertoire T cells develop severe, multiorgan, Th2-associated inflammatory disease. *Proc Natl Acad Sci U S A* Jan 9;2007 104(2):576–81. [PubMed: 17202252]

28. Turul T, Tezcan I, Artac H, de Bruin-Versteeg S, Barendregt BH, Reisli I, et al. Clinical heterogeneity can hamper the diagnosis of patients with ZAP70 deficiency. *Eur J Pediatr* Jan;2009 168(1):87–93. [PubMed: 18509675]
29. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, et al. Combined immunodeficiency associated with DOCK8 mutations. *N Engl J Med* Nov 19;2009 361(21):2046–55. [PubMed: 19776401]
30. Dupre L, Aiuti A, Trifari S, Martino S, Saracco P, Bordignon C, et al. Wiskott-Aldrich syndrome protein regulates lipid raft dynamics during immunological synapse formation. *Immunity* Aug; 2002 17(2):157–66. [PubMed: 12196287]
31. Randall KL, Lambe T, Johnson AL, Treanor B, Kucharska E, Domaschensz H, et al. Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. *Nat Immunol* Dec;2009 10(12):1283–91. [PubMed: 19898472]
32. Sasahara Y, Rachid R, Byrne MJ, de la Fuente MA, Abraham RT, Ramesh N, et al. Mechanism of recruitment of WASP to the immunological synapse and of its activation following TCR ligation. *Mol Cell* Dec;2002 10(6):1269–81. [PubMed: 12504004]
33. Gerwien J, Nielsen M, Labuda T, Nissen MH, Svejgaard A, Geisler C, et al. Cutting edge: TCR stimulation by antibody and bacterial superantigen induces Stat3 activation in human T cells. *J Immunol* Aug 15;1999 163(4):1742–5. [PubMed: 10438903]